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=> d history

     (FILE 'HOME' ENTERED AT 14:23:48 ON 28 NOV 2001)

     FILE 'MEDLINE, BIOSIS, LIFESCI' ENTERED AT 14:24:26 ON 28 NOV 2001
L1         50601 S NITRIC OXIDE SYNTHASE
L2          6010 S ENOS OR NNOS OR CNOS
L3          2280 S (AUTOINHIBIT? OR (AUTO-INHIBIT?))
L4            59 S L1 AND L3
L5             6 S L4 AND PY<1997
L6            28 S L2 AND L3
L7             0 S L6 AND PY<1997
L8             3 DUP REM L5 (3 DUPLICATES REMOVED)
L9         51039 S L1 OR L2
L10         8522 S (AGONI? OR ACTIVAT?)(S)L9
L11         2463 S L10 AND PY<1997
L12         8522 S L10(5A)L9
L13         1861 S L1(5A)(AGONI? OR ACTIVAT?)
L14          577 S L2(5A)(AGONI? OR ACTIVAT?)
L15         2225 S L13 OR L14
L16          539 S L15 AND PY<1997
L17         6871 S L1(3A)(NEUR? OR NERV? OR BRAIN)
L18         7680 S L1(3A)(ENDOTHEL? OR CONSTITUTIVE)
L19          752 S L17(S)(AGONI? OR ACTIVAT?)
L20          971 S L18(S)(AGONI? OR ACTIVAT?)
L21         1859 S L14 OR L19 OR L20
L22          406 S L21 AND PY<1997
L23          200 DUP REM L22 (206 DUPLICATES REMOVED)

=> log h
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
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     FILE 'REGISTRY' ENTERED AT 17:57:24 ON 28 NOV 2001
L1            39 S AGMATINE
L2            14 S HARMALINE
L3            33 S FLAVIN ADENINE DINUCLEOTIDE
L4             5 S SOD

     FILE 'MEDLINE, BIOSIS, LIFESCI' ENTERED AT 18:38:45 ON 28 NOV 2001
L5         18212 S (BETA(A)AMYLOID) OR (B(A)AMYLOID) OR BAMYLOID OR AMYLOIDB
L6         50615 S NITRIC OXIDE SYNTHASE
L7          3854 S L6(A)(NERVE OR NEURON? OR BRAIN)
L8           717 S CNOS
L9            15 S L8(A)(NERVE OR NEURON? OR BRAIN)
L10         2913 S NNOS OR BNOS
L11         5074 S L10 OR L9 OR L7
L12          734 S L11(S)(ACTIVA? OR AGONI?)
L13           14 S L12(S)L5
L14            0 S L13 AND PY<1997
L15        11669 S L6(S)(NERVE OR NEURON? OR BRAIN)
L16          192 S L8(S)(NERVE OR NEURON? OR BRAIN)
L17        12285 S L15 OR L16 OR L10
L18         2491 S L17(S)(ACTIVA? OR AGONI?)
L19           46 S L18(S)L5
L20            8 S L19 AND PY<1997
L21            3 DUP REM L20 (5 DUPLICATES REMOVED)

     FILE 'SCISEARCH' ENTERED AT 18:51:26 ON 28 NOV 2001
L22            0 S (BETA-AMLYOID)/TI
L23         2067 S (BETA-AMYLOID)/TI
L24          775 S 4/RVL(S)760/RPG
L25            7 S L23 AND L24
L26           23 S 4/RVL(S)760/RPG(S)HU?/RAU
L27            8 S L26 AND PY<1997

     FILE 'MEDLINE, BIOSIS, LIFESCI' ENTERED AT 19:56:39 ON 28 NOV 2001
L28          536 S L11(S)STIMULAT?
L29         5086 S ENOS OR ENDOTHELI?(A)L6
L30          735 S L29(S)STIMULAT?
L31         1148 S L28 OR L30
L32          168 S L31 AND PY<1997
L33           80 S L11(5A)STIMULAT?
L34          244 S L29(5A)STIMULAT?
L35          322 S L33 OR L34
L36           45 S L35 AND PY<1997
L37           23 DUP REM L36 (22 DUPLICATES REMOVED)

=> log h
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WEST

Help | Logout | Interrupt

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences

Search Results -

Terms	Documents
134 or 135	15

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: 134 or 135 Clear

Search History

Today's Date: 11/28/2001

DB Name	Query	Hit Count	Set Name
USPT	134 or 135	15	L36
USPT	133 and @prad<19960712	5	L35
USPT	133 and @ad<19960712	10	L34
USPT	15 with stimulat$5	36	L33
DWPI	129 and (stimul$5)	6	L32
DWPI	129 and (agoni$4 or activat$$)	13	L31
DWPI	129 and ((auto or self) adj (regulat$4 or control$4 or inhibit$4))	0	L30
DWPI	125 or 126 or 127 or 128	118	L29
DWPI	eNOS or (endotheli$2 adj nitric adj oxide adj synthase)	80	L28
DWPI	bNOS or nNOS	18	L27
DWPI	(nerv$3 or brain or neuron$2) near2 (NOS or cNOS)	21	L26
DWPI	(nerv$3 or brain or neuron$2) near2 (nitric adj oxide adj synthase)	16	L25

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JPAB,EPAB	l19 or l20 or l21 or l22	85	L23
JPAB,EPAB	eNOS or (endotheli$2 adj nitric adj oxide adj synthase)	62	L22
JPAB,EPAB	bNOS or nNOS	9	L21
JPAB,EPAB	(nerv$3 or brain or neuron$2) near2 (NOS or cNOS)	2	L20
JPAB,EPAB	(nerv$3 or brain or neuron$2) near2 (nitric adj oxide adj synthase)	16	L19
JPAB,EPAB,DWPI	l14 or l15 or l16 or l17	203	L18
JPAB,EPAB,DWPI	eNOS or (endotheli$2 adj nitric adj oxide adj synthase)	142	L17
JPAB,EPAB,DWPI	bNOS or nNOS	27	L16
JPAB,EPAB,DWPI	(nerv$3 or brain or neuron$2) near2 (NOS or cNOS)	23	L15
JPAB,EPAB,DWPI	(nerv$3 or brain or neuron$2) near2 (nitric adj oxide adj synthase)	32	L14
USPT	l12 or l11	37	L13
USPT	l6 and @prad<19960712	11	L12
USPT	l6 and @ad<19960712	29	L11
USPT	l5 with (auto-inhibit$4)	0	L10
USPT	l5 with autocrine	0	L9
USPT	l5 with (auto-regulat$4)	0	L8
USPT	l5 with (autoinhibit$4 or (anto-inhibit$4) or (anti-regulat$4) or (autoregulat$4))	2	L7
USPT	l5 with (agoni$4 or activ$5)	94	L6
USPT	l1 or l2 or l3 or l4	543	L5
USPT	eNOS or (endotheli$2 adj nitric adj oxide adj synthase)	381	L4
USPT	bNOS or nNOS	98	L3
USPT	(nerv$3 or brain or neuron$2) near2 (NOS or cNOS)	105	L2
USPT	(nerv$3 or brain or neuron$2) near2 (nitric adj oxide adj synthase)	131	L1

DOCUMENT-IDENTIFIER: US 5919682 A
**** See image for Certificate of Correction ****
TITLE: Overproduction of neuronal nitric oxide synthase

Abstract Text (1):
The present invention is directed to overproduction of nitric oxide synthase (NOS) in a prokaryote. More particularly, the invention involves overexpression of functional neuronal NOS in a protease-deficient strain of Escherichia coli, utilizing a pCW vector under the control of the tac promotor. The invention further involves co-expression of the protein with folding agonists, or chaperonins. The enzyme produced is complete with heme and flavins, and may be activated by incubation with tetrahydrobiopterin. It may be isolated as a predominantly high spin heme protein that demonstrates spectral properties which are identical to those of nNOS isolated from human kidney 293 cells. The methods disclosed are contemplated to be useful in expressing large amounts of other nitric oxide synthases, as well as other proteins that are difficult to produce correctly folded in prokaryotes.

Brief Summary Text (19):
Also disclosed herein are methods of producing nitric oxide synthase, comprising first obtaining a protease-deficient prokaryotic cell that comprises a first nucleotide sequence that encodes a nitric oxide synthase, and a second nucleotide sequence that encodes a folding agonist. Each of the nucleotide sequences is under the control of a promoter, which may be an inducible or constitutive promoter, and the cells may be grown in the presence of heme precursor (.delta.-aminolevulinate) and flavin precursors. Nitric oxide synthase apoenzyme is isolated from the cell, and incubated with tetrahydrobiopterin for activation from apoenzyme to holoenzyme.

Brief Summary Text (22):
It also is recognized that other large heme, flavin or other prosthetic group-containing proteins could be expressed using a combination of the techniques disclosed herein. For example, heme and flavin biosynthetic precursors are included in the present invention for nNOS expression.

Detailed Description Text (19):
Protein expression. Fernbach flasks containing 1 liter of modified Terrific Broth (20 g yeast extract, 10 g bactotryptone, 2.65 g KH.sub.2 PO.sub.4, 4.33 g Na.sub.2 HPO.sub.4, 4 ml glycerol) and 50 .mu.g/ml ampicillin and, when pGroELS was present, 35 .mu.g/ml chloramphenicol were inoculated with 1 ml of an overnight culture (grown in LB+antibiotics) and shaken at 250 rpm at 37.degree. C. Protein expression was induced at OD.sub.600 =1.0-1.4 with the addition of IPTG to 0.5 mM. The heme and flavin precursors, .delta.-aminolevulinic acid and riboflavin, were also added to final concentrations of 450 .mu.M and 3 .mu.M, respectively. When pGroELS was present, ATP to 1 mM was also added to the culture media. The flasks were moved to room temperature (25.degree. C.) and shaken in the dark at 250 rpm. The cells were harvested at about 40 hours post-induction in the cell paste frozen at -80.degree. C. until purification.

Detailed Description Text (29):
Spectral characteristics of purified E. coli-expressed enzyme. FIG. 1A shows the absolute spectrum of nNOS isolated from E. coli. It exhibits a broad peak at 400 nm and secondary maxima at 550 and 650 nm, indicative of a predominantly high spin heme, although some low spin form is present, as evidenced by the shoulder at 410

nm. Shoulders are also apparent at 450 and 475 nm and are due to flavin absorbance; this spectrum is identical to that of nNOS isolated from human kidney 293 cells (McMillan et al., 1992). As shown in FIG. 2, the maximum heme absorbance at 400 nm can be shifted to the low spin form (peak at 428 nm) by the addition of imidazole to 1 mM or completely to the high spin form (peak at 395 nm) by the addition of arginine to 2 .mu.M.

Other Reference Publication (31):
Mayer et al., "Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase", FEBS Letters, 288(1,2):187-191, Aug. 1991.

Other Reference Publication (33):
McMillian et al., "Prokaryotic Expression of the Heme- and Flavin-Binding Domains of Rat Neuronal Nitric Oxide Synthase as Distinct Polypeptides: Identification of the Heme-Binding Proximal Thiolate Ligand as Cysteine-415", Biochemistry, 34:3686-3693, 1995.

CLAIMS:

1. A method of producing nitric oxide synthase comprising:

(i) obtaining a protease-deficient prokaryotic cell, the cell comprising a first nucleotide sequence that encodes a nitric oxide synthase, and a second nucleotide sequence that encodes a chaperonin that is in the class of GroEL and GroES;

(ii) growing the cells in the presence of heme precursor (d-aminolevulinate) or flavin precursors; and

(iii) isolating nitric oxide synthase from the cell.

2. A method of producing nitric oxide synthase comprising:

(i) obtaining a protease-deficient prokaryotic cell, the cell comprising a first nucleotide sequence that encodes a nitric oxide synthase, and a second nucleotide sequence that encodes a chaperonin that is in the class of GroEL and GroES;

(ii) growing the cells in the presence of heme precursor (d-aminolevulinate) and flavin precursors; and

(iii) isolating nitric oxide synthase apoenzyme from the cell.

L14: Entry 1 of 7 File: USPT Nov 21, 2000

US-PAT-NO: 6150500
DOCUMENT-IDENTIFIER: US 6150500 A

TITLE: Activators of endothelial nitric oxide synthase

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Salerno; John C.	Troy	NY	12180	

US-CL-CURRENT: 530/300; 530/326, 530/327, 530/387.1, 530/387.9, 530/388.2, 530/388.26

CLAIMS:

What is claimed is:

1. An extrinsic activator of endothelial nitric oxide synthase which antagonizes autoinhibition by interacting with an intrinsic peptide region of endothelial nitric oxide synthase, wherein the region consists of amino acids 590-650 of endothelial nitric oxide synthase (SEQ ID NO. 30, aa 78-138).

2. An antibody, or fragment thereof, which binds to an epitope comprising amino acids between about amino acids 590-650 in the regulatory region of endothelial nitric oxide synthase (SEQ ID NO. 30, aa 78-138).

3. The antibody of claim 2, wherein the antibody activates endothelial nitric oxide synthase.

4. A constitutive nitric oxide synthase activator peptide consisting of an amino acid sequence selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, and activating fragments and derivatives of SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9 in which the activator function of the parent compound is conserved.

5. An activator of endothelial nitric oxide synthase which binds to amino acids in a sequence selected from the group consisting of: SEQ ID NO. 21 and SEQ ID NO. 24.

6. A constitutive nitric oxide synthase activator peptide consisting of an amino acid sequence selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and SEQ ID NO. 9.

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L1: Entry 1 of 1 File: USPT Nov 21, 2000

US-PAT-NO: 6150500
DOCUMENT-IDENTIFIER: US 6150500 A

TITLE: Activators of endothelial nitric oxide synthase

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Salerno; John C.	Troy	NY	12180	

US-CL-CURRENT: 530/300; 530/326, 530/327, 530/387.1, 530/387.9, 530/388.2, 530/388.26

CLAIMS:

What is claimed is:
1. An extrinsic activator of endothelial nitric oxide synthase which antagonizes autoinhibition by interacting with an intrinsic peptide region of endothelial nitric oxide synthase, wherein the region consists of amino acids 590-650 of endothelial nitric oxide synthase (SEQ ID NO. 30, aa 78-138).
2. An antibody, or fragment thereof, which binds to an epitope comprising amino acids between about amino acids 590-650 in the regulatory region of endothelial nitric oxide synthase (SEQ ID NO. 30, aa 78-138).
3. The antibody of claim 2, wherein the antibody activates endothelial nitric oxide synthase.
4. A constitutive nitric oxide synthase activator peptide consisting of an amino acid sequence selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, and activating fragments and derivatives of SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9 in which the activator function of the parent compound is conserved.
5. An activator of endothelial nitric oxide synthase which binds to amino acids in a sequence selected from the group consisting of: SEQ ID NO. 21 and SEQ ID NO. 24.
6. A constitutive nitric oxide synthase activator peptide consisting of an amino acid sequence selected from the group consisting of: SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and SEQ ID NO. 9.

08/679,006

L34 ANSWER 4 OF 12 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 96386458 MEDLINE
DOCUMENT NUMBER: 96386458 PubMed ID: 8794208
TITLE: Understanding the structural aspects of neuronal **nitric oxide synthase** (NOS) using microdissection by molecular cloning techniques: molecular dissection of neuronal NOS.
AUTHOR: Masters B S; McMillan K; Nishimura J; Martasek P; Roman L J; Sheta E; Gross S S; **Salerno J**
CORPORATE SOURCE: Department of Biochemistry, The University of Texas Health Science Center at San Antonio, 78284-7760, USA.
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, **(1996)** 387 163-9.
Journal code: 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970114

L34 ANSWER 3 OF 12 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 96376740 MEDLINE
DOCUMENT NUMBER: 96376740 PubMed ID: 8782612
TITLE: **Nitric oxide synthases**: analogies to cytochrome P450 monooxygenases and characterization of recombinant rat neuronal **nitric oxide synthase** hemoprotein.
AUTHOR: McMillan K; **Salerno J C**; Masters B S
CORPORATE SOURCE: Pharmacopeia, Cranbury, New Jersey 08512, USA.
CONTRACT NUMBER: HL30050 (NHLBI)
SOURCE: METHODS IN ENZYMOLOGY, **(1996)** 268 460-72.
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970305
Last Updated on STN: 19970305
Entered Medline: 19970218

L10 ANSWER 15 OF 23 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 94012662 MEDLINE
DOCUMENT NUMBER: 94012662 PubMed ID: 7691806
TITLE: Macrophage nitric oxide synthase subunits. Purification, characterization, and role of prosthetic groups and substrate in regulating their association into a dimeric enzyme.
AUTHOR: Baek K J; Thiel B A; Lucas S; Stuehr D J
CORPORATE SOURCE: Department of Immunology, Cleveland Clinic, Ohio 44195.
CONTRACT NUMBER: CA53914 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1993 Oct 5)** 268 (28) 21120-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19960129
Entered Medline: 19931124

AB The cytokine-**induced nitric oxide synthase** (NOS) of macrophages is a homodimeric enzyme that contains iron protoporphorin IX (heme), FAD, **FMN**, tetrahydrobiopterin, and calmodulin. To investigate how the enzyme's quaternary structure relates to its catalytic activity and binding of prosthetic groups, dimeric NOS and its subunits were purified separately and their composition and catalytic properties compared. In contrast to dimeric NOS, purified subunits did not synthesize NO or contain bound heme or tetrahydrobiopterin. However, the subunits did contain FAD, FMN, and calmodulin in amounts comparable with dimeric NOS, displayed the light absorbance spectrum of an FAD- and FMN-containing flavoprotein, and generated an air-stable flavin semiquinone radical upon reduction of their ferricyanide-oxidized form. Dimeric NOS and NOS subunits were equivalent in catalyzing electron transfer from NADPH to cytochrome c, dichlorophenolindophenol, or ferricyanide at rates that were 8-30-fold faster than the maximal rate of NO synthesis by dimeric NOS. Reconstitution of subunit NO synthesis required their incubation with L-arginine, tetrahydrobiopterin, and stoichiometric amounts of heme and correlated with formation of a proportional amount of dimeric NOS in all cases. The dimeric NOS reconstituted from its subunits contained 0.9 heme and 0.44 tetrahydrobiopterin bound per subunit and had the spectral and catalytic properties of native dimeric NOS. Thus, NOS subunits are NADPH-dependent reductases that acquire the capacity to synthesize NO only through their dimerization and binding of heme and tetrahydrobiopterin. The ability of heme, tetrahydrobiopterin, and L-arginine to promote subunit dimerization is unprecedented and suggests novel roles for these molecules in forming and stabilizing the active dimeric NOS.

L24 ANSWER 7 OF 19 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 95096039 MEDLINE
DOCUMENT NUMBER: 95096039 PubMed ID: 7528206
TITLE: Calmodulin controls neuronal **nitric-oxide synthase** by a dual mechanism. **Activation** of intra- and interdomain electron transfer.
AUTHOR: Abu-Soud H M; Yoho L L; Stuehr D J
CORPORATE SOURCE: Department of Immunology, Cleveland Clinic, Ohio 44195.
CONTRACT NUMBER: CA53914 (NCI)
GM51491 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 Dec 23)** 269 (51) 32047-50.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19960129
Entered Medline: 19950124

AB In neuronal nitric-oxide synthase (NOS), electron transfer proceeds across domains in a linear sequence from NADPH to **flavins** to heme, with calmodulin (CaM) triggering the interdomain electron transfer to the heme (Abu-Soud, H. M., and Stuehr, D. J. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10769-10772). Here, we utilized a neuronal NOS devoid of its bound heme and tetrahydrobiopterin (apo-NOS) to examine whether interdomain electron transfer is responsible for CaM's activation of NO synthesis, substrate-independent NADPH oxidation, and cytochrome c and ferricyanide reduction. Of the four activities, two (cytochrome c and ferricyanide reduction) were similarly stimulated by CaM in apo-NOS when compared with native NOS, indicating that activation occurs by a mechanism not involving **flavin**-to-heme electron transfer. Further analysis showed that CaM increased the rate of electron transfer from NADPH into the **flavin** centers by a factor of 20, revealing a direct activation of the NOS reductase domain by CaM. In contrast, CaM's activation of NO synthesis and substrate-independent NADPH oxidation appeared to involve **flavin**-to-heme electron transfer because these reactions were not activated in apo-NOS and were blocked in native NOS by agents that prevent heme iron reduction. Thus, CaM activates neuronal NOS at two points in the electron transfer sequence: electron transfer into the **flavins** and interdomain electron transfer between the **flavins** and heme. Activation at each point is associated with an up-regulation of domain-specific catalytic functions. The dual regulation by CaM is unique and represents a new means by which electron transfer can be controlled in a metalloflavoprotein.

L10 ANSWER 1 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

ACCESSION NUMBER: 1996:466272 BIOSIS
DOCUMENT NUMBER: PREV199699188628
TITLE: Characterization of the reductase domain of rat neuronal nitric oxide synthase generated in the methylotrophic yeast Pichia pastoris: Calmodulin response is complete within the reductase domain itself.
AUTHOR(S): Gachhui, Ratan; Presta, Anthony; Bentley, Dennis F.; Abu-Soud, Husam M.; McArthur, Ryan; Brudvig, Gary; Ghosh, Dipak K.; Stuehr, Dennis J. (1)
CORPORATE SOURCE: (1) Immunol. NN-1, Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH 44195 USA
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 34, pp. 20594-20602.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Rat neuronal NO synthase (nNOS) is comprised of a **flavin** -containing reductase domain and a heme-containing oxygenase domain. Calmodulin binding to nNOS increases the rate of electron transfer from NADPH into its **flavins**, triggers electron transfer from **flavins** to the heme, activates NO synthesis, and increases reduction of artificial electron acceptors such as cytochrome c. To investigate what role the reductase domain plays in calmodulin's activation of these functions, we overexpressed a form of the nNOS reductase domain (amino acids 724-1429) in the yeast Pichia pastoris that for the first time exhibits a complete calmodulin response. The reductase domain was purified by 2',5'-ADP affinity chromatography yielding 25 mg of pure protein per liter of culture. It contained 1 FAD and 0.8 **FMN** per molecule. Most of the protein as isolated contained an air-stable **flavin** semiquinone radical that was sensitive to FeCN-6 oxidation. Anaerobic titration of the FeCN-6-oxidized reductase domain with NADPH indicated the **flavin** semiquinone re-formed after addition of 1-electron equivalent and the **flavins** could accept up to 3 electrons from NADPH. Calmodulin binding to the recombinant reductase protein increased its rate of NADPH-dependent **flavin** reduction and its rate of electron transfer to cytochrome c, FeCN-6, or dichlorophenolindophenol to fully match the rate increases achieved when calmodulin bound to native full-length **nNOS**. Calmodulin's **activation** of the reductase protein was associated with an increase in domain tryptophan and **flavin** fluorescence. We conclude that many of calmodulin's actions on native nNOS can be fully accounted for through its interaction with the nNOS reductase domain itself.

L10 ANSWER 4 OF 23 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 96223636 MEDLINE
DOCUMENT NUMBER: 96223636 PubMed ID: 8634274
TITLE: Domains of macrophage N(O) synthase have divergent roles in forming and stabilizing the active dimeric enzyme.
AUTHOR: Ghosh D K; Abu-Soud H M; Stuehr D J
CORPORATE SOURCE: Department of Immunology, Cleveland Clinic Research Institute, Ohio 44195, USA.
CONTRACT NUMBER: CA53914 (NCI)
SOURCE: BIOCHEMISTRY, **(1996 Feb 6)** 35 (5) 1444-9.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960719
Last Updated on STN: 19960719
Entered Medline: 19960705

AB The cytokine-**inducible** NO synthase (**iNOS**) is a **flavin**-containing hemeprotein that must dimerize to generate NO. Trypsin cleaves the dimeric enzyme into an oxygenase domain fragment that remains dimeric, contains heme and H4biopterin, and binds L-arginine and a reductase domain fragment that is monomeric, binds NADPH, FAD, FMN, and catalyzes the reduction of cytochrome c [Ghosh, D. K. & Stuehr, D. J. (1995) Biochemistry 34, 801-807]. The current study investigates the isolated oxygenase and reductase domains of iNOS to understand how they form and stabilize the active dimeric enzyme. The dimeric oxygenase domain dissociated into folded, heme-containing monomers when incubated with 2-5 M urea, whereas the reductase domain unfolded under these conditions and lost its ability to catalyze NADPH-dependent cytochrome c reduction. Spectral analysis of the dissociation reaction showed that it caused structural changes within the oxygenase domain and exposed the distal side of the heme to solvent, enabling it to bind dithiothreitol as a sixth ligand. Importantly, the oxygenase domain monomers could reassociate into a dimeric form even in the absence of the reductase domain. The reaction required L-arginine and H4biopterin and completely reversed the structural changes in heme pocket and protein structure that occurred upon dissociating the original dimer. Together, this confirms that the oxygenase domain contains all of the determinants needed for subunit dimerization and indicates that the dimeric structure greatly affects the heme and protein environment in the oxygenase domain.

L10 ANSWER 15 OF 23 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 94012662 MEDLINE
DOCUMENT NUMBER: 94012662 PubMed ID: 7691806
TITLE: Macrophage nitric oxide synthase subunits. Purification, characterization, and role of prosthetic groups and substrate in regulating their association into a dimeric enzyme.
AUTHOR: Baek K J; Thiel B A; Lucas S; Stuehr D J
CORPORATE SOURCE: Department of Immunology, Cleveland Clinic, Ohio 44195.
CONTRACT NUMBER: CA53914 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1993 Oct 5)** 268 (28) 21120-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19960129
Entered Medline: 19931124

AB The cytokine-**induced nitric oxide synthase** (NOS) of macrophages is a homodimeric enzyme that contains iron protoporphorin IX (heme), FAD, **FMN**, tetrahydrobiopterin, and calmodulin. To investigate how the enzyme's quaternary structure relates to its catalytic activity and binding of prosthetic groups, dimeric NOS and its subunits were purified separately and their composition and catalytic properties compared. In contrast to dimeric NOS, purified subunits did not synthesize NO or contain bound heme or tetrahydrobiopterin. However, the subunits did contain FAD, FMN, and calmodulin in amounts comparable with dimeric NOS, displayed the light absorbance spectrum of an FAD- and FMN-containing flavoprotein, and generated an air-stable flavin semiquinone radical upon reduction of their ferricyanide-oxidized form. Dimeric NOS and NOS subunits were equivalent in catalyzing electron transfer from NADPH to cytochrome c, dichlorophenolindophenol, or ferricyanide at rates that were 8-30-fold faster than the maximal rate of NO synthesis by dimeric NOS. Reconstitution of subunit NO synthesis required their incubation with L-arginine, tetrahydrobiopterin, and stoichiometric amounts of heme and correlated with formation of a proportional amount of dimeric NOS in all cases. The dimeric NOS reconstituted from its subunits contained 0.9 heme and 0.44 tetrahydrobiopterin bound per subunit and had the spectral and catalytic properties of native dimeric NOS. Thus, NOS subunits are NADPH-dependent reductases that acquire the capacity to synthesize NO only through their dimerization and binding of heme and tetrahydrobiopterin. The ability of heme, tetrahydrobiopterin, and L-arginine to promote subunit dimerization is unprecedented and suggests novel roles for these molecules in forming and stabilizing the active dimeric NOS.

L10 ANSWER 8 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8

ACCESSION NUMBER: 1995:479734 BIOSIS
DOCUMENT NUMBER: PREV199598494034
TITLE: Reconstitution of the second step in NO synthesis using the isolated oxygenase and reductase domains of macrophage NO synthase.
AUTHOR(S): Ghosh, Dipak K.; Abu-Soud, Husam M.; Stuehr, Dennis J. (1)
CORPORATE SOURCE: (1) Immunol. NN-1, Cleveland Clin., 9500 Euclid Ave., Cleveland, OH 44195 USA
SOURCE: Biochemistry, (1995) Vol. 34, No. 36, pp. 11316-11320. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English

AB **Inducible** macrophage NO synthase (**iNOS**) is a homodimer of 130 kDa subunits. Trypsinolysis of iNOS inactivates its NO synthesis activity and cleaves the enzyme into a dimeric oxygenase fragment that contains heme, tetrahydrobiopterin, and the substrate binding site and a monomeric reductase fragment that contains FAD, **FMN**, calmodulin, and the binding site for NADPH (Ghosh, D. I., & Stuehr, D. H. (1995) Biochemistry 34, 801-807). In this paper, we describe the reconstitution of NO synthesis activity utilizing the isolated oxygenase and reductase domains of iNOS. Mixing the domains at various ratios showed that NO was not produced from L-arginine but could be formed from the reaction intermediate N-omega-hydroxy-L-arginine (L-NOHA). The apparent K-m with L-NOHA in the reconstituted system was 100 mu-M versus 19 mu-M for native iNOS. D-NOHA was not a substrate. Maximum specific activity (per heme) occurred at an oxygenase to reductase molar ratio of 4:1, with higher ratios causing some inhibition. Reconstitution of activity was associated with electron transfer between the domain fragments and led to an incomplete reduction of the oxygenase domain heme iron. L-NOHA, but not L-arginine, increased NADPH consumption in the reconstituted system. Between 2.5 and 3.0 NADPH were consumed per NO formed from L-NOHA, considerably higher than the stoichiometry obtained with native iNOS (0.5 NADPH oxidized per NO formed), indicating an uncoupled electron transfer between the domain fragments. Thus, the isolated iNOS reductase and oxygenase domains each retain their separate catalytic functions but interact to catalyze only the second step of NO synthesis. In this way, they form a monooxygenase system similar to those of eukaryotic cytochromes P-450.

L10 ANSWER 7 OF 23 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96195111 MEDLINE
DOCUMENT NUMBER: 96195111 PubMed ID: 8660310
TITLE: Identification of the domains of neuronal nitric oxide synthase by limited proteolysis.
AUTHOR: Lowe P N; Smith D; Stammers D K; Riveros-Moreno V; Moncada S; Charles I; Boyhan A
CORPORATE SOURCE: Biology Division, GlaxoWellcome Research Laboratories, Beckenham, Kent, U.K.
SOURCE: BIOCHEMICAL JOURNAL, **(1996 Feb 15)** 314 (Pt 1) 55-62.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960808
Last Updated on STN: 19960808
Entered Medline: 19960726

AB Nitric oxide synthase (EC 1.14.13.39) binds arginine and NADPH as substrates, and FAD, FMN, tetrahydrobiopterin, haem and calmodulin as cofactors. The protein consists of a central calmodulin-binding sequence flanked on the N-terminal side by a haem-binding region, analogous to cytochrome P-450, and on the C-terminal side by a region homologous with NADPH:cytochrome P-450 reductase. The structure of recombinant rat brain nitric oxide synthase was analysed by limited proteolyis. The products were identified by using antibodies to defined sequences, and by N-terminal sequencing. Low concentrations of trypsin produced three fragments, similar to those in a previous report [Sheta, McMillan and Masters (1994) J. Biol. Chem. 269, 15147-15153]: that of Mr approx. 135000 (N-terminus Gly-221) resulted from loss of the N-terminal extension (residues 1-220) unique to neuronal nitric oxide synthase. The fragments of Mr 90000 (haem region) and 80000 (reductase region, N-terminus Ala-728) were produced by cleavage within the calmodulin-binding region. With more extensive trypsin treatment, these species were shown to be transient, and three smaller, highly stable fragments of Mr 14000 (N-terminus Leu-744 within the calmodulin region), 60000 (N-terminus Gly-221) and 63000 (N-terminus Lys-856 within the FMN domain) were formed. The species of Mr approx. 60000 represents a domain retaining haem and nitroarginine binding. The two species of Mr 63000 and 14000 remain associated as a complex. This complex retains cytochrome c reductase activity, and thus is the complete reductase region, yet cleaved at Lys-856. This cleavage occurs within a sequence insertion relative to the **FMN** domain present in **inducible nitric oxide synthase**. Prolonged proteolysis treatment led to the production of a protein of Mr approx. 53000 (N-terminus Ala-953), corresponding to a cleavage between the FMN and FAD domains. The major products after chymotryptic digestion were similar to those with trypsin, although the pathway of intermediates differed. The haem domain was smaller, starting at residue 275, yet still retained the arginine binding site. These data have allowed us to identify stable domains representing both the arginine/haem-binding and the reductase regions.

L10 ANSWER 12 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11

ACCESSION NUMBER: 1995:532208 BIOSIS
DOCUMENT NUMBER: PREV199598546508
TITLE: Nitric oxide synthase: Expression and expressional control of the three isoforms.
AUTHOR(S): Forstermann, Ulrich (1); Kleinert, Hartmut
CORPORATE SOURCE: (1) Dep. Pharmacol., Johannes Gutenberg Univ., Obere Zahlbacher Strasse 67, D-55101 Mainz Germany
SOURCE: Naunyn-Schmiedeberg's Archives of Pharmacology, (1995) Vol. 352, No. 4, pp. 351-364.
ISSN: 0028-1298.
DOCUMENT TYPE: General Review
LANGUAGE: English

AB Three isozymes of nitric oxide synthase (NOS) have been identified. Their cDNA- and protein structures as well as their genomic DNA structures have been described. NOS I (ncNOS, originally discovered in neurons) and NOS III (ecNOS, originally discovered in endothelial cells) are low output, Ca-2+activated enzymes whose physiological function is signal transduction. NOS II (**iNOS**, originally discovered in cytokine-**induced** macrophages) is a high output enzyme which produces toxic amounts of NO that represent an important component of the antimicrobial, antiparasitic and antineoplasic activity of these cells. Depending on the species, NOS II activity is largely (human) or completely (mouse and rat) Ca-2+independent. In the human species, the NOS isoforms I, II and III are encoded by three different genes located on chromosomes 12, 17 and 7, respectively. The amino acid sequences of the three human isozymes (deduced from the cloned cDNAs) show less than 59% identity. Across species, amino acid sequences are more than 90% conserved for NOS I and III, and greater 80% identical for NOS II. All NOS produce NO by oxidizing a guanidino nitrogen of L-arginine utilizing molecular oxygen and NADPH as co-substrates. All isoforms contain FAD, **FMN** and heme iron as prosthetic groups and require the cofactor BH-4. NOS I and III are constitutively expressed in various cells. Nevertheless, expression of these isoforms is subject to regulation. Expression is enhanced by e.g. estrogens (for NOS I and III), shear stress, TGF-beta-1, and (in certain endothelial cells) high glucose (for NOS III). TNF-alpha reduces the expression of NOS III by a post-transcriptional mechanism destabilizing the mRNA. The regulation of the NOS I expression seems to be very complex as reflected by at least 8 different promoters transcribing 8 different exon 1 sequences which are expressed differently in different cell types. Expression of NOS II is mainly regulated at the transcriptional level and can be induced in many cell types with suitable agents such as LPS, cytokines, and other compounds. Whether some cells can express NOS It constitutively is still under debate. Pathways resulting in the induction of the NOS II promoter may vary in different cells. Activation of transcription factor NF-kappa-B seems to be an essential step for NOS II induction in most cells. The induction of NOS II can be inhibited by a wide variety of immunomodulatory compounds acting at the transcriptional levels and/or post-transcriptionally.

L45 ANSWER 31 OF 46 MEDLINE DUPLICATE 19

ACCESSION NUMBER: 95104455 MEDLINE
DOCUMENT NUMBER: 95104455 PubMed ID: 7528687
TITLE: Inducible **nitric oxide synthase** in human lymphomononuclear cells **activated** by synthetic **peptides** derived from extracellular matrix proteins.
AUTHOR: Perez-Mediavilla L A; Lopez-Zabalza M J; Calonge M; Montuenga L; Lopez-Moratalla N; Santiago E
CORPORATE SOURCE: Department of Biochemistry, University of Navarra, Pamplona, Spain.
SOURCE: FEBS LETTERS, **(1995 Jan 3)** 357 (2) 121-4.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19960129
Entered Medline: 19950202

AB Synthetic peptides with sequences present in extracellular matrix proteins are capable of causing the expression of the inducible form of nitric oxide synthase (iNOS), detected by immunocytochemistry, and the release of NO by human lymphomononuclear cells incubated in their presence. Active peptides are 15-mers containing a characteristic 2-6-11 motif in which the amino acid residue at position 2 is Leu, Ile, Val, Gly, Ala or Lys; the residue at position 6 is always Pro; and residue 11 is Glu or Asp. The induction of iNOS in human monocytes and macrophages could be involved in the cytotoxicity against tumor cell lines also elicited by these peptides.

L24 ANSWER 4 OF 19 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 96114752 MEDLINE
DOCUMENT NUMBER: 96114752 PubMed ID: 7492972
TITLE: **Activation** of neuronal **nitric oxide synthase** by **flavin** adenine dinucleotide.
AUTHOR: Hashida-Okumura A; Nagai K; Okumura N; Nakagawa H
CORPORATE SOURCE: Division of Protein Metabolism, Osaka University, Japan.
SOURCE: BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, **(1995 May)** 35 (6) 1339-48.
Journal code: 9306673. ISSN: 1039-9712.
PUB. COUNTRY: Australia
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19970203
Entered Medline: 19960111

AB Bovine adrenals were found to contain a factor that activates neuronal nitric oxide synthase (NOS) and reduces the blood pressure when injected into the lateral cerebral ventricle (LCV). This factor showed chemical and functional characteristics similar to **flavin** adenine dinucleotide (FAD). Therefore, the effect of FAD on neuronal NOS activity was examined. FAD caused at least 2-fold stimulation of NOS partially purified from rat brain. This effect was not simply due to formation of the holoenzyme. Kinetic analyses showed that NOS exhibited negative cooperativity with L-arginine, its substrate, and FAD counteracted this effect. Furthermore, injection of FAD into the LCV reduced the blood pressure. These results suggest that FAD stimulates neuronal NOS by counteracting its negative cooperativity with L-arginine and also lowers the blood pressure by activating NOS.

L10 ANSWER 17 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15

ACCESSION NUMBER: 1993:429519 BIOSIS
DOCUMENT NUMBER: PREV199396084144
TITLE: Autoinhibition of murine macrophage-mediated oxidation of low-density lipoprotein by nitric oxide synthesis.
AUTHOR(S): Jessup, Wendy (1); Dean, Roger T.
CORPORATE SOURCE: (1) Heart Res. Inst., 145 Missenden Road, Sydney, NSW 2050 Australia
SOURCE: Atherosclerosis, (1993) Vol. 101, No. 2, pp. 145-155. ISSN: 0021-9150.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Murine peritoneal macrophages treated with gamma-interferon and lipopolysaccharide (activated cells) oxidized low-density lipoprotein (LDL) less readily than unstimulated cells. **Activated** cells expressed the enzyme **nitric oxide synthase**, whose activity was measured by the accumulation of nitrite in the culture supernatant. Treatment of activated macrophages with the arginine analogue N-G-monomethyl-arginine (NMMA) inhibited nitric oxide synthesis and restored the ability of the cells to oxidize LDL. This treatment had no effect on the ability of unstimulated cells to oxidize LDL. Similarly, LDL oxidation by activated macrophages in arginine-free Ham's F-10 medium was identical to that of unstimulated cells, whereas restoration of arginine to the medium was associated with nitrite secretion and a decline in LDL oxidation by activated cells only. An inverse relationship between nitric oxide synthesis and LDL oxidation was also demonstrated in the presence of diphenylene iodonium, a **flavin** analogue which is a potent inhibitor of nitric oxide synthase. Thus nitric oxide synthesis appears to mediate the suppression of LDL oxidation which is associated with the activation of mouse macrophages by gamma-interferon and lipopolysaccharide.

L10 ANSWER 16 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14

ACCESSION NUMBER: 1993:298430 BIOSIS
DOCUMENT NUMBER: PREV199396016655
TITLE: Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes.
AUTHOR(S): Geller, David A. (1); Lowenstein, Charles J.; Shapiro, Richard A.; Nussler, Andreas K.; Di Silvio, Mauricio; Wang, Stewart C.; Nakayama, Don K.; Simmons, Richard L.; Snyder, Solomon H.; Billar, Timothy R.
CORPORATE SOURCE: (1) Dep. Surgery, Univ. Pittsburgh, Pittsburgh, PA 15261 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993) Vol. 90, No. 8, pp. 3491-3495.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Nitric oxide is a short-lived biologic mediator for diverse cell types. Synthesis of an **inducible nitric oxide synthase** (NOS) in murine macrophages is stimulated by lipopolysaccharide (LPS) and interferon gamma. In human hepatocytes, NOS activity is induced by treatment with a combination of tumor necrosis factor, interleukin 1, interferon gamma, and LPS. We now report the molecular cloning and expression of an inducible human hepatocyte NOS (hep-NOS) cDNA. hep-NOS has 80% amino acid sequence homology to macrophage NOS (mac-NOS). Like other NOS isoforms, recognition sites for **FMN**, FAD, and NADPH are present, as well as a consensus calmodulin binding site. NOS activity in human 293 kidney cells transfected with hep-NOS cDNA is diminished by Ca-2+ chelation and a calmodulin antagonist, reflecting a Ca-2+ dependence not evident for mac-NOS. Northern blot analysis with hep-NOS cDNA reveals a 4.5-kb mRNA in both human hepatocytes and aortic smooth muscle cells following stimulation with LPS and cytokines. Human genomic Southern blots probed with human hep-NOS and human endothelial NOS cDNA clones diaplay different genomic restriction enzyme fragments, suggesting distinct gene products for these NOS isoforms. hep-NOS appears to be an inducible form of NOS that is distinct from mac-NOS as well as brain and endothelial NOS isozymes.

L45 ANSWER 45 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 30

ACCESSION NUMBER: 1993:345959 BIOSIS
DOCUMENT NUMBER: PREV199396042959
TITLE: Vasoactive intestinal peptide release and L-citrulline production from isolated ganglia of the myenteric plexus: Evidence for regulation of vasoactive intestinal peptide release by nitric oxide.
AUTHOR(S): Grider, J. R. (1); Jin, J.-G.
CORPORATE SOURCE: (1) Box 711, MCV Station, Med. Coll. Va., Richmond, VA 23298-0711 USA
SOURCE: Neuroscience, (1993) Vol. 54, No. 2, pp. 521-526. ISSN: 0306-4522.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Vasoactive intestinal peptide release and L-(3H)citrulline production were examined in ganglia isolated from the myenteric plexus of guinea-pig intestine. The nicotinic agonist, 1,1-dimethyl-4-phenylpiperizinium **stimulated** vasoactive intestinal **peptide** release and L-(3H)citrulline production; the latter was considered an index of nitric oxide production. Both vasoactive intestinal peptide release and L-(3H)citrulline production were abolished by tetrodotoxin, hexamethonium, and the **nitric oxide synthase** inhibitor, N-G-nitro-L-arginine. Inhibition of vasoactive intestinal peptide release by N-G-nitro-L-arginine was reversed by L-arginine but not by D-arginine. Exogenous nitric oxide **stimulated** vasoactive intestinal **peptide** release whereas exogenous vasoactive intestinal peptide had no effect on L-(3H)citrulline production. The pattern of stimulation by nitric oxide and inhibition by N-G-nitro-L-arginine implied that vasoactive intestinal peptide release is facilitated by and may be dependent on nitric oxide production. Consistent with this notion, vasoactive intestinal peptide release in response to either 1,1-dimethyl-4-phenylpiperizinium or nitric oxide was abolished by KT 5823, an inhibitor of cyclic GMP-dependent protein kinase activity and by LY83583, an inhibitor of soluble guanylate cyclase activity. The study provides the first direct evidence of nitric oxide production from enteric ganglia.

L45 ANSWER 43 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 28

ACCESSION NUMBER: 1995:38638 BIOSIS
DOCUMENT NUMBER: PREV199598052938
TITLE: Regulation of the descending relaxation phase of intestinal peristalsis by PACAP.
AUTHOR(S): Grider, J. R. (1); Katsoulis, S.; Schmidt, W. E.; Jin, J.-G.
CORPORATE SOURCE: (1) Dep. Physiol., Med. Coll. Va., Box 980711, Richmond, VA 23298 USA
SOURCE: Journal of the Autonomic Nervous System, (1994) Vol. 50, No. 2, pp. 151-159.
ISSN: 0165-1838.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The presence of pituitary adenylate cyclase-**activating peptide** (PACAP), a homologue of vasoactive intestinal peptide (VIP), in enteric neurons suggests that it may be involved in the regulation of the descending relaxation phase of the peristaltic reflex. The role of PACAP was evaluated by measurement of PACAP release and by immuno-neutralization with specific monoclonal antibodies to PACAP-27 and PACAP-38, and an antibody to VIP. Electrical field stimulation at 4 Hz caused a 12-fold increase in PACAP release that was inhibited by 53 +- 6% (P lt 0.01) by the **nitric oxide synthase** inhibitor, N-G-nitro-L-arginine (L-NNA). Orad stretch of colonic segments elicited descending relaxation and PACAP release in proportion to the degree of stretch. PACAP release induced by 10-g stretch was inhibited by 67 +- 10% (P lt 0.01) by L-NNA. Previous studies (Am. J. Physiol., 264 (1993) G334-G340) showed that orad stretch elicits also VIP release and nitric oxide (NO) production and that VIP release is inhibited (59%) by L-NNA. Preincubation of the segments with PACAP-27 plus PACAP-38 antibodies (50 mu-g/ml each), or with VIP antibody (1:60) inhibited descending relaxation at all degrees of stretch (inhibition with PACAP antibodies: 90 +- 8% with 2-g and 22 +- 5% with 10-g stretch). Preincubation with both PACAP and VIP antibodies virtually abolished descending relaxation. A similar pattern was observed with the antagonists, PACAP6-38 and VIP10-28, alone and in combination. Studies in dispersed colonic muscle cells showed that (i) VIP- and PACAP-induced relaxation was inhibited by each antagonist and by L-NNA, and (ii) selective receptor protection with each peptide preserved the response to all three peptides, implying interaction of PACAP and VIP with a common receptor coupled to NO generation in muscle cells. We conclude that PACAP and VIP are the main determinants of descending relaxation and that their effect reflects interplay with NO in neurons and muscle cells.

L45 ANSWER 35 OF 46 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:295712 CAPLUS
DOCUMENT NUMBER: 122:72396
TITLE: CGRP enhances induction of NO synthase in vascular smooth muscle cells via a cAMP-dependent mechanism
AUTHOR(S): Schini-Kerth, V. B.; Fisslthaler, B.; Busse, R.
CORPORATE SOURCE: Zentrum der Physiologie, Johann-Wolfgang-Goeth-Universitaet, Frankfurt, D-60590, Germany
SOURCE: American Journal of Physiology (1994), 267(6, Pt. 2), H2483-H2490
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Expts. were designed to examine whether calcitonin gene-related peptide (CGRP), a potent cAMP-dependent vasodilator, affects the prodn. of NO evoked by interleukin-1.beta. (IL-1.beta.) in cultured rat aortic smooth muscle cells (SMC). CGRP, in a concn.-dependent manner, enhanced the release of nitrite (a stable oxidn. product of NO) and the formation of L-citrulline from L-arginine caused by IL-1.beta.. Two cAMP-dependent vasodilators, forskolin and isoproterenol, and the activator of the cAMP-dependent protein kinase, Sp-cAMPS, also enhanced the release of nitrite and the formation of L-citrulline evoked by IL-1.beta.. The enhancing effect of isoproterenol required the presence of the vasodilator during the induction of NO synthase (NOS). IL-1.beta.-treated vascular SMC inhibited the aggregation of indomethacin-treated platelets. Inhibition of platelet aggregation was more marked with SMC exposed to a combination of IL-1.beta. and either CGRP or isoproterenol than with cells exposed to IL-1.beta. alone. This inhibition was prevented by methylene blue and oxyHb. IL-1.beta. induced the expression of inducible NOS mRNA in vascular SMC, which was enhanced by coincubation of IL-1.beta. with either CGRP, isoproterenol, or forskolin. These observations indicate that CGRP via a cAMP-dependent mechanism potentiates the IL-1.beta.-induced prodn. of NO by enhancing the expression of inducible NOS. Therefore CGRP may contribute to the substantial prodn. of NO in the vasculature during septic shock, which accounts, at least in part, for the collapse of the vascular system.

L45 ANSWER 34 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 22

ACCESSION NUMBER: 1995:81710 BIOSIS
DOCUMENT NUMBER: PREV199598096010
TITLE: The dendritic peptide neurogranin can regulate a calmodulin-dependent target.
AUTHOR(S): Martzen, Mark R.; Slemmon, J. Randall (1)
CORPORATE SOURCE: (1) Dep. Biochem., Box 607, University Rochester Med. Cent., 601 Elmwood Ave., Rochester, NY 14642 USA
SOURCE: Journal of Neurochemistry, (1995) Vol. 64, No. 1, pp. 92-100.
ISSN: 0022-3042.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Neurogranin, a peptide capable of binding the calcium-poor form of calmodulin, was tested in vitro for its ability to modulate a typical calmodulin target. The target employed was the calcium/calmodulin-dependent form of **nitric oxide-synthase**, which is produced by several different types of neurons. Neurogranin for the study was purified from perchloric acid-soluble calf brain proteins by a combination of calmodulin-Sepharose affinity chromatography and reverse-phase HPLC. The protocol yielded highly purified neurogranin that was active in assays using purified **nitric oxide synthase**. The titration of the enzyme activity with neurogranin demonstrated a concentration-dependent effect of the **peptide** on enzyme **activation**. Subsequent analysis of the ability of increased calcium concentrations to activate the enzyme was performed in the presence of different amounts of neurogranin. The effect of neurogranin on the calcium-dependent activation of the enzyme was to depress enzyme activity in the range of 0.2 to apprx 1 mu-M calcium. Treatment of the neurogranin peptide with protein kinase C eliminated its inhibition on **nitric oxide synthase** activation. Treatment of the protein kinase C-phosphorylated peptide with calcineurin did not restore the ability of neurogranin to inhibit enzyme activity, whereas treatment with alkaline phosphatase did restore this ability. These results suggest that neurogranin may serve as a member of a unique class of endogenous calmodulin inhibitor that functions to regulate the activation of calmodulin-requiring targets in neurons.

L45 ANSWER 26 OF 46 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:139213 CAPLUS
DOCUMENT NUMBER: 124:242000
TITLE: Induction of macrophage NO-synthase by an immunomodulator entrapped within polymeric nanocapsules
AUTHOR(S): Seyler, I.; Barratt, G.; Appel, M.; Devissaguet, J. P.; Puisieux, F.
CORPORATE SOURCE: Faculte de Pharmacie, Universite de Paris-Sud, Chatenay-Malabry, 92296, Fr.
SOURCE: World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 1st, Budapest, May 9-11, 1995 (**1995**), 521-2. APGI: Chatenay Malabry, Fr.
CODEN: 62JJAQ
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The comparison of muramyldipeptide with muramyl tripeptide-cholesterol entrapped within biodegradable nanocapsules in a in vitro model consisting of a mouse macrophage line RAW 264.7 is studied. The conditions under which muramyl **peptides** can **induce nitric oxide synthase** activity in these cells are described.

L45 ANSWER 22 OF 46 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 95237144 MEDLINE
DOCUMENT NUMBER: 95237144 PubMed ID: 7536663
TITLE: Natriuretic **peptide**-augmented **induction** of **nitric oxide synthase** through cyclic guanosine 3',5'-monophosphate elevation in vascular smooth muscle cells.
AUTHOR: Marumo T; Nakaki T; Hishikawa K; Hirahashi J; Suzuki H; Kato R; Saruta T
CORPORATE SOURCE: Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.
SOURCE: ENDOCRINOLOGY, **(1995 May)** 136 (5) 2135-42.
Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950605
Last Updated on STN: 19990129
Entered Medline: 19950523

AB To elucidate the role of natriuretic peptides in vascular remodeling, the effects of atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic **peptide** (CNP) on the **induction** of inducible nitric oxide (NO) synthase (**iNOS**) in rat aortic smooth muscle cells were examined. Although none of the peptides when applied alone induced the production of nitrite, a stable end product of NO, each peptide dramatically enhanced nitrite production induced by a cytokine combination of interleukin-1 alpha and tumor necrosis factor-alpha. Each natriuretic peptide stimulated intracellular cGMP accumulation in a dose-dependent manner. Time-dependent nitrite production by the cytokines was increased by CNP cotreatment and inhibited by NG-methyl-L-arginine, indicating involvement of the L-arginine-NO pathway. Northern blot analysis showed that the augmented nitrite production was accompanied by an increase in iNOS messenger RNA. A cGMP analog, 8-bromo-cGMP, completely mimicked all of the effects of CNP described above. A cGMP-dependent protein kinase inhibitor, KT5823, paradoxically increased nitrite production and iNOS messenger RNA levels induced by the combination of 8-bromo-cGMP and both cytokines or by the two cytokines only. These data demonstrate the stimulatory effect of cGMP on cytokine-induced iNOS and imply that natriuretic peptides may play a regulatory role in vascular remodeling via the production of large amounts of NO.

L45 ANSWER 17 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8

ACCESSION NUMBER: 1996:410731 BIOSIS
DOCUMENT NUMBER: PREV199699133087
TITLE: Vasoactive intestinal **peptide** (VIP)-**induced** enzyme secretion in rat pancreatic tissue is not associated with activation of **nitric oxide synthase** (NOS) and increase in cyclic GMP level.
AUTHOR(S): Nam, Tae Kyun; Han, Jeung Whan; Nam, Suk Woo; Seo, Dong-Wan; Lee, Young Jin; Ko, Young Kwon; Lee, Hyang Woo (1)
CORPORATE SOURCE: (1) Coll. Pharmacy, Sungkyunkwan Univ., Suwon 440-746 South Korea
SOURCE: Archives of Pharmacal Research (Seoul), (1996) Vol. 19, No. 3, pp. 201-206.
ISSN: 0253-6269.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Nitric oxide (NO) is thought to be a second messenger involved in secretion. Upon stimulating pancreatic acinar cells with cholecystokinin-pancreozymin (CCK-PZ), NO formation has been shown to be associated with increased levels of cGMP (Seo et al., 1995). To elucidate the signaling pathway of VIP-induced enzyme secretion, we investigated the NO and cGMP synthesis steps as potential steps where two signal pathways triggered by CCK-PZ and VIP interact. The results obtained in this work provide evidence that increase in pancreatic enzyme secretion by treatment with VIP has no relationship with NOS activity and cGMP level. This conclusion was derived from the following findings that VIP treatment of rat pancreatic tissue increased amylase release as well as protein output in a dose- and time-dependent manner, whereas NOS activity and cGMP synthesis were not affected by VIP treatment as monitored by NOS activity assay and determining cGMP level, which was further confirmed by a NOS-inhibitor study. Consequently, CCK-PZ or VIP increases enzyme secretion in rat pancreatic tissue, but the two hormones are different in their mode of action. Together the results suggest that signaling pathway of VIP-induced enzyme secretion might either bypass the NO and cGMP synthesis steps or lie on a distinct pathway from CCK-PZ-induced pathway.

L45 ANSWER 16 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:450445 BIOSIS
DOCUMENT NUMBER: PREV199699172801
TITLE: Natriuretic **peptide**-augmented **induction** of **nitric oxide synthase** through cyclic GMP elevation in vascular smooth muscle cells.
AUTHOR(S): Marumo, Takeshi (1); Nakaki, Toshio; Hishikawa, Keiichi; Hirahashi, Junichi; Kato, Ryuichi; Saruta, Takao
CORPORATE SOURCE: (1) Dep. Pharmacol., Keio Univ. Sch. Med., 35 Shinanomachi, Shinjuku-ku, Tokyo 160 Japan
SOURCE: Japanese Journal of Pharmacology, (1996) Vol. 71, No. SUPPL. 1, pp. 316P.
Meeting Info.: 69th Annual Meeting of the Japanese Pharmacological Society Nagasaki, Japan March 20-23, 1996
ISSN: 0021-5198.
DOCUMENT TYPE: Conference
LANGUAGE: English

L45 ANSWER 7 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4

ACCESSION NUMBER: 1996:220725 BIOSIS
DOCUMENT NUMBER: PREV199698776854
TITLE: Neuropeptides induce release of nitric oxide from human dermal microvascular endothelial cells.
AUTHOR(S): Bull, Helen A. (1); Hothersall, John; Chowdhury, Nazira; Cohen, John; Dowd, Pauline M.
CORPORATE SOURCE: (1) Division Med., Univ. Coll. London Med. Sch., The Rayne Institute, 5 University St., London WC1E 6JJ UK
SOURCE: Journal of Investigative Dermatology, (1996) Vol. 106, No. 4, pp. 655-660.
ISSN: 0022-202X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Nitric oxide is a potent mediator of endothelium-dependent vasodilatation, the synthesis of which is catalyzed by the constitutively expressed enzyme endothelial **nitric oxide synthase**. In this study we have investigated whether human dermal microvascular endothelial cells express endothelial **nitric oxide synthase** and whether the vasodilator neuropeptides, calcitonin gene-related **peptide** and substance P, **stimulate** the release of nitric oxide from these cells. Endothelial **nitric oxide synthase** was identified by immunohistochemistry in the blood vessels in both the papillary and deep dermis of normal skin, and also in monolayers of human dermal microvascular endothelial cells. On western blots of protein extracts prepared from both the dermis of normal human skin and human dermal microvascular endothelial cells, a 135-kDa band corresponding to endothelial **nitric oxide synthase** was identified. Nitric oxide was released from unstimulated human dermal microvascular endothelial cells as assessed by inhibition of platelet aggregation and nitrite formation. Endothelial cell-mediated inhibition of platelet aggregation was blocked by hemoglobin, which binds nitric oxide. Substance P (10 nM) potentiated microvascular endothelial cell inhibition of platelet aggregation, and this effect was also blocked by hemoglobin. Calcitonin gene-related peptide (100 pM to 100 nM) directly inhibited platelet aggregation, and this direct effect was not modulated by microvascular endothelial cells. Substance P (10 nM to 1 mu-M) and calcitonin gene-related peptide (100 pM to 10 nM) significantly (p lt 0.05) increased nitrite formation, and this increase was blocked by the competitive **nitric oxide synthase** antagonist, N-G-monomethyl-L-arginine. These results demonstrate that endothelial **nitric oxide synthase** is expressed in the microvascular endothelium of normal human skin and that human dermal microvascular endothelial cells release nitric oxide constitutively and in response to vasodilator neuropeptides.

L45 ANSWER 6 OF 46 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

ACCESSION NUMBER: 1996:153820 BIOSIS
DOCUMENT NUMBER: PREV199698725955
TITLE: Regulation of interleukin-1-beta-stimulated inducible nitric oxide synthase expression in cultured vascular smooth muscle cells by hemostatic proteins.
AUTHOR(S): Durante, William (1); Kroll, Michael H.; Orloff, Gregory J.; Cunningham, James M.; Scott-Burden, Timothyh; Vanhoutte, Paul M.; Schafer, Andrew I.
CORPORATE SOURCE: (1) Houston VA Med. Center, Build. 109, Room 116, 2002 Holcombe Blvd., Houston, TX 77030 USA
SOURCE: Biochemical Pharmacology, (1996) Vol. 51, No. 6, pp. 847-853.
ISSN: 0006-2952.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Experiments were performed to examine the mechanism by which specific hemostatic proteins regulate the release of nitric oxide (NO) from interleukin-1-beta (IL-1-beta) stimulated cultured rat aortic smooth muscle cells. Treatment of smooth muscle cells with IL-beta stimulated inducible **nitric oxide synthase** (**iNOS**) mRNA expression, which preceded the release of NO (as measured by the accumulation of nitrite in the culture media). The cytokine-stimulated production of nitrite was blocked by the protein synthesis inhibitor cycloheximide, the transcriptional inhibitor actinomycin D, and the competitive inhibitor of NOS nitro-L-arginine. However, only actinomycin D inhibited IL-1-beta-stimulated **iNOS** mRNA expression. Treatment of smooth muscle cells with IL-1-beta in the presence of platelet derived growth factor or thrombin resulted in the inhibition of cytokine-stimulated expression of **iNOS** mRNA and NO release. The inhibitory effect of thrombin was reversed by hirudin and was mimicked by a 14 amino acid thrombin receptor **activating peptide**. In contrast, the concomitant exposure of smooth muscle cells to IL-1-beta and plasmin resulted in the potentiation of both IL-1-beta-stimulated **iNOS** expression and NO generation. Finally, treatment of smooth muscle cells with IL-1-beta in the presence of the hemostatic proteins did not affect the half-life of **iNOS** mRNA. These results demonstrate that specific protein components of the hemostatic system regulate IL-1-beta-stimulated **iNOS** mRNA expression in vascular smooth muscle cells. The capacity of hemostatic proteins to modulate the induction of vascular **iNOS** activity may play an important role in governing the release of NO and regulating thrombogenesis in vivo.

L45 ANSWER 5 OF 46 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:465241 CAPLUS
DOCUMENT NUMBER: 125:133488
TITLE: Functional evidence for NO-synthase activation by substance P through a mechanism not involving classical tachykinin receptors in guinea pig ileum in vitro
AUTHOR(S): Garcia-Villar, R.; Dupuis, C.; Martinolle, J. P.; Fioramonti, J.; Bueno, L.
CORPORATE SOURCE: INRA, Pharmacology & Toxicology Unit, Toulouse, 31931, Fr.
SOURCE: British Journal of Pharmacology (1996), 118(5), 1253-1261
CODEN: BJPCBM; ISSN: 0007-1188
PUBLISHER: Stockton
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This study tested the hypothesis that a nitric oxide synthase (NOS) was activated in guinea pig ileum in vitro in response to substance P (SP), and attempted to characterize the tachykinin receptor involved in this activation by the use of selective receptor agonists and antagonists. Strips of guinea pig ileum (8 .times. 2 mm) were superfused (Krebs, 37.degree., 2 mL min-1) with: (i) tachykinin receptor agonists: SP, GR 73632 (NK1), GR 64349 (NK2), senktide (NK3), and neuropeptide (NP).gamma.; (ii) tachykinin receptor antagonists: CP 99994 (NK1), SR 48968 (NK2), SR 142801 (NK3); (iii) nerve-related agents: carbachol (CCh), atropine, tetrodotoxin (TTX), hexamethonium; (iv) NOS inhibitors: N.omega.-nitro-L-arginine Me ester (L-NAME), N.omega.-monomethyl-L-arginine (L-NMMA) and aminoguanidine (AG); (v) NO-related agents, L-arginine (L-Arg), D-arginine (D-Arg), sodium nitroprusside (NaNP) and metHb. Muscle contractility was recorded isometrically and quantified as integrated area of activity. SP, tachykinin receptor agonists and NP.gamma. (10 pM to 10 .mu.M), produced concn.-dependent contractions of ileal strips, with EC50s in the nanomolar range, and maximal responses (Emax) attained at 0.1 .mu.M for SP and 1 .mu.M for the other agonists. The Emax response to SP equaled that to KCl (60 nM) taken as a 100% control (99.3%); a comparable Emax contraction was obtained with the other tachykinin receptor agonists (1 .mu.M) as well as with CCh (1 .mu.M). Under baseline conditions, L-NAME (1 .mu.M), L-NMMA (1 .mu.M) and AG (1 .mu.M), failed to contract the muscle strip. In contrast, when superfused for 3 min, 10 min after SP (0.1 .mu.M), they induced a transient contraction of the strip (e.g. for 1 .mu.M L-NAME: 50 to 70 s duration; amplitude 73%). The NOS inhibitor-induced contractile response was not obtained after KCl (60 nM), GR 73632, GR 64349, senktide or CCh (all up to 1 .mu.M). In contrast, this contractile response was obtained after NP.gamma. (1 .mu.M). Blockade of tachykinin NK1, NK2 and NK3 receptors by continuous superfusion of CP 99994, SR 48968 and SR 142801 (1 .mu.M) resp., starting 5 min before SP, did not modify the response to L-NAME, superfused 10 min after SP (0.1 .mu.M). The contractile response to L-NAME (1 .mu.M) was blocked by atropine (1 .mu.M), superfused either before or after SP. In contrast, it persisted after TTX or hexamethonium (1 .mu.M) superfused in the same conditions. The amplitude of NOS inhibitor-induced contraction (1 .mu.M) was dependent on the concn. of priming SP (1 pM to 1 .mu.M). In contrast, the contractile response to NOS inhibitors (1 nM to 10 .mu.M) of the ileum strip primed with SP (0.1 .mu.M) was not concn.-related. L-NAME-induced contraction was prevented by continuous superfusion of L-Arg (1 .mu.M), but not D-Arg (1 .mu.M). In addn., the NO donor, sodium nitroprusside (1 .mu.M) and the NO scavenger, metHb (10 .mu.g mL-1), both prevented the contractile response to L-NAME. In summary, SP and to a lesser extent NP.gamma., exert a permissive action allowing contractile stimulating effects of L-NAME, L-NMMA and AG, in guinea pig ileum in vitro, by a mechanism which apparently does not involve tachykinin NK1, NK2 and NK3 receptors. This action is likely to

result from the activation of a NO-synthase by SP in the vicinity of intestinal myocytes. Thus, L-NAME, L-NMMA or AG, by blocking this SP-induced NO prodn., unveiled a smooth muscle contraction which involves a cholinoceptor (atropine-sensitive) mechanism.

L45 ANSWER 4 OF 46 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:733268 CAPLUS
DOCUMENT NUMBER: 126:27269
TITLE: Adrenomedullin augments inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes
AUTHOR(S): Ikeda, Uichi; Kanbe, Toshiko; Kawahara, Yasuhiro; Yokoyama, Mitsuhiro; Shimada, Kazuyuki
CORPORATE SOURCE: Department Cardiology, Jichi Medical School, Tochigi, 329-04, Japan
SOURCE: Circulation (1996), 94(10), 2560-2565
CODEN: CIRCAZ; ISSN: 0009-7322
PUBLISHER: American Heart Association
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Plasma levels of adrenomedullin are increased in patients with congestive heart failure, but there has been no report concerning the effects of adrenomedullin on the heart. The authors investigated the effects of adrenomedullin on NO synthase activity in cardiac myocytes. The authors measured the prodn. of nitrite, a stable metabolite of NO, in cultured neonatal rat cardiac myocytes with the Griess reagent. Inducible NO synthase mRNA and protein expression were assayed by Northern and Western blotting, resp. Incubation of the cultures with interleukin-1.beta. (10 ng/mL) for 24 h caused a significant increase in nitrite accumulation. Adrenomedullin significantly augmented nitrite prodn. by interleukin-1.beta.-stimulated but not by unstimulated cardiac myocytes in a dose-dependent manner (10-10 to 10-6 M). The adrenomedullin-induced nitrite prodn. by interleukin-1.beta.-stimulated cells was accompanied by increased inducible NO synthase mRNA and protein expression. In the presence of dibutyryl cAMP, the interleukin-1.beta.-induced nitrite accumulation was increased further, but the stimulatory effect of adrenomedullin on nitrite prodn. was abolished. Adrenomedullin dose-dependently increased intracellular cAMP levels in cardiac myocytes. Addn. of the calcitonin gene-related peptide (CGRP) receptor antagonist CGRP[8-37] to the culture dose-dependently inhibited both cAMP and NO generation stimulated by adrenomedullin. These results indicate that adrenomedullin acts on cardiac myocytes and augments NO synthesis in these cells under cytokine-stimulated conditions, at least partially through a cAMP-dependent pathway.